PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07H 5/06 — A1

(11) International Publication Number: WO 97/00879

(43) International Publication Date: 9 January 1997 (09.01.97)

(21) International Application Number: PCT/SE96/00824

(22) International Filing Date: 20 June 1996 (20.06.96)

(30) Priority Data:
9502264-6 21 June 1995 (21.06.95) SE

(71) Applicant *(for all designated States except US)*: ISM, INSTITUTE FOR SOCIO-MEDICAL RESEARCH [CH/CH]; Case postale 718, CH-2001 Neuchâtel (CH).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: DAHLGREN, Åke [SE/CH]; Chemin des Sauges 14, CH-2015 Areuse (CH). DAHLGREN, Atti-La [SE/CH]; Chemin des Sauges 14, CH-2015 Areuse (CH). KISS, Laszlo [HU/HU]; Simony út 32, H-4028 Debrecen (HU).

(74) Agents: IVERSEN HASSELROT, Eva et al.; L. A. Groth & Co. KB, P.O. Box 6107, S-102 32 Stockholm (SE).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: METHOD FOR PREPARING SULPHATE ESTERS EFFECTIVE IN THE TREATMENT OF HIV (HUMAN IMMUNODEFICIENCY VIRUS) INFECTIONS, AND AIDS

(57) Abstract

The present invention relates to a method for preparing sulphate esters of pentoses and hexoses and their salts, together with sulphur trioxide-triethylamine complex to form a mono-, di-, tri- or tetra-sulphate ester of the pentose or hexose.

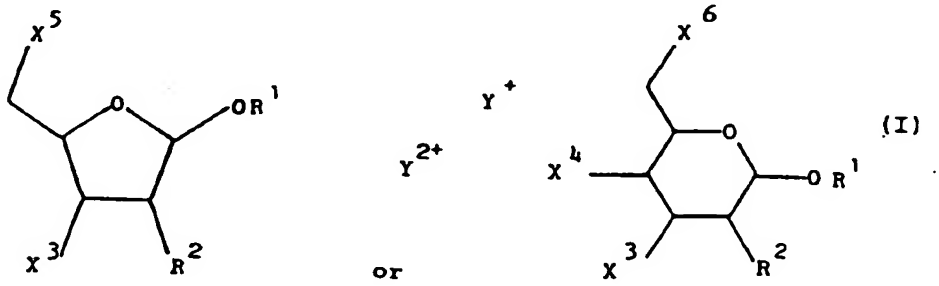
FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgystan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic			SE	Sweden
CG	Congo	KR	Republic of Korea	SG	Singapore
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LR	Liberia	SZ	Swaziland
CS	Czechoslovakia	LT	Lithuania	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	LV	Latvia	TJ	Tajikistan
DK	Denmark	MC	Monaco	TT	Trinidad and Tobago
EE	Estonia	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	UG	Uganda
FI	Finland	ML	Mali	US	United States of America
FR	France	MN	Mongolia	UZ	Uzbekistan
GA	Gabon	MR	Mauritania	VN	Viet Nam

Method for preparing sulphate esters effective in the treatment of HIV (Human Immuno Deficiency Virus) infections, and AIDS.

The present invention relates to a method for preparing mono-, di-, tri-or tetra-sulphate esters of pentoses and hexoses and their salts, see formulas (I).



(I)

Where

R^1 is hydrogen, C_1-C_6-alkyl, benzyl, amino acid, nucleotide, or polypeptide;

R^2 is OH, SO_4^- or NHR^3;

R^3 is hydrogen, C_1-C_6-alkyl, acetyl or C_2-C_{24}-acyl, aminoacyl or sulphonyl;

X^3 is OH or SO_4^-;

X^4 is OH or SO_4^-;

X^5 is OH or SO_4^-;

X^6 is OH or SO_4^-;

Y^+, Y^{2+} is H, Na, K, Ca, Zn, Mg, Li, Ba, Mn, Hg, Ag or Au.

Background of the Invention

Sulphate esters of hexosamines have been prepared by direct sulphation of hexosamines with chlorosulphonic acid. Previously, Lloyd, A.G. (1962) Biochem. J. 83, 455-460 and Suzuki, S. and Strominger, J.L. (1960) J. Biol. Chem. 235, 267-273 showed that sulphate esters prepared by the direct sulphation of N-acetyllgalactosamine with chlorosulfonic acid consists of mixtures of mono-and bisulphate esters of the sugar. In a later publication Kazuhiko Ishihara et al. Biochim. Biophys. Acta, 437 (1979) 416-430, synthesised N-acetylgalactosamine-4,6-disulphate. UDP-GalNAc-4-sulphate was treated with chlorosulfonic acid, the disulphated nucleotide thus prepared was hydrolysed with 0.01 M HCl to result in N-acetylgalactosamine-4,6-disulphate. In

an other paper Yasuo Nakanishi et al. J. of Biol. Chem. vol. 256 No. 11 (1981) 5443-5449, sulphate was introduced into position 6 of the nonreducing terminal N-acetylgalactosamine-4-sulphate by a terminal 6-sulphotransferase.

Description of the Invention

The general method for preparing sulphated pentoses and hexoses according to the invention include sulphation with sulphur trioxide-triethylamine complex of hydroxyl-protected sugars in a polar solvent, such as N,N-dimethyl formamide at 20-80°C, preferably 30-70°C, most preferably 40-60°C. This reaction step is very effective and yield almost quantitative yields in mild conditions, it is also very efficient in producing specially designed sulphate esters.

The sulphation method according to the invention also include catalytic hydrogenation in presence of a catalyst, such as palladium hydroxide, to give compounds with formulas (I).

One embodiment of the present invention is the method for preparation of sulphate ester derivatives of N-acetyl-D-galactosamine. The method comprises treatment of N-acetyl-D-galactosamine with benzyl alcohol and Amberlite® IR 120 to yield benzyl-N-acetyl-D-galactosaminide. The hydroxyl groups of benzyl-N-acetyl-D-galactosaminide was protected and treated with sulphur trioxide-triethylamine complex in N,N-dimethyl formamide at 20-80°C. Depending on which hydroxyl groups were protected the reaction yielded mono-, di- or tri-sulphate substituted benzyl-N-acetyl-D-galactosaminide. The sulphated galactosaminide was catalytically hydrogenated with palladium hydroxide and was commonly worked up to give 50-99% of mono-, di- or tri-sulphate substituted N-acetyl-D-galactosamine.

A further embodiment of the present invention is a method for preparation of sulphate ester derivatives of N-acetyl-D-glucosamine. The preparation procedure followed the same route as described above and yielded after

common workup procedures 50-99% of mono-, di- or tri-sulphate substituted N-acetyl-D-glucosamine.

The following examples are given by way of example the invention only and not by way of limitation thereof.

Example 1

3,4,6-tri-O-sulpho-N-acetyl-D-galactosamine

A solution of benzyl-N-acetyl-D-galactosaminide (155mg) was stirred in N,N-dimethyl formamide (2 ml) for 15 hours at 50°C in presence of sulphur trioxide-triethylamine complex (380mg). The mixture was cooled and methanol (1 ml) was added. The mixture was chromatographed on a silica gel (30 g) column with ethylacetate:pyridine:acetic acid:water (8:5:1:3) to yield a pure fraction, this was solved in methanol (1 ml) and was further eluated from a Sephadex® SP 25 (Na^+-form 2x25 cm) column with methanol:water (1:9) to afford the 3,4,6-tri-O-sulpho-derivative (220 mg, 80%). The benzyl-groups were removed in a solution of ethanol:water (2:1) and catalytic hydrogenation in presence of palladium hydroxide (20% Pd) for 1 day. The suspension was filtered and concentrated, finally lyophilised. SO calc. 64.86% found 63.5%.

Example 2

3,4-di-sulpho-N-acetyl-D-galactosamine

A solution of benzyl 4,6-O-benzylidene-N-acetyl-D-galactosaminide (1 mmol) and sodium cyanoborhydride (9 mmol) in dry THF (15 ml) containing powdered 3A molecular sieves was cooled to 0°C. Hydrogen chloride in diethyl ether was added dropwise until the solution was acidic (pH paper, gas evolution). The mixture was monitored with TLC and when completed (after 10 min. at 0°C) it was poured into ice-water. The product was extracted with dichloromethane and purified on a silica gel column. The 6-O-benzyl derivative (278mg, 85%) was O-sulphated as described in example 1 to give 3,4-di-sulpho-N-acetyl-D-

galactosamine (75mg, 91%). SO calc. 50.65% found 49.0%.

Example 3

4,6-di-D-sulpho-N-acetyl-D-galactosamine

A solution of benzyl-4,6-O-benzylidene-N-acetyl-D-galactosaminide (400 mg) in dry N,N-dimethylformamide (6 ml) was stirred at 0°C in presence of barium oxide (845,5 mg), barium hydroxide 8 H_2O (261 mg) and benzyl bromide (232 µl) was added. After completion of the reaction (TLC, dichloromethane:methanol (95:5)) the excess of benzyl bromide was quenched with methanol (150 µl). The mixture was diluted with chloroform, washed with water, dried (Na_2SO_4) and concentrated. The residue was crystallized from ethanol yielding 472 mg (70%) of product. The benzylidene group was removed by treatment of the product (400mg) with 60% acetic acid in water (10ml) with stirring at 60 C for 3 hours, then cooled, and concentrated. The acetic acid was removed by repeated evaporation with toluene and 295 mg (90%) of 3-O-benzyl derivative was obtained. This compound was O-sulphated as described in example 1 and gave 74,8 mg (83%) of 4,6-di-O-sulpho-N-acetyl-D-galactosamine. SO calcd. 50.65%, Found 49.5%.

Example 4

4-O-sulpho-N-acetyl-D-galactosamine

A solution of benzyl-4,6-O-benzylidene-N-acetyl-D-galactosaminide (400 mg) was treated as described in example 3. The di-O-benzyl derivative was O-sulphated as described in example 1 to give 4-O-sulpho-N-acetyl-D-galactosamine (180mg, 91%). NMR data: 2,1 ppm s N-Ac; 3,6-4 ppm m skeleton H; 4,1-4,2 ppm m C^6-H; 4,6 ppm d C^4-H; 5,2 ppm d anomer H. The NMR spectra were recorded on a Brucker 200 instrument. SO calc. 32.0% found 30.6%.

Example 5

3-sulpho-N-acetyl D-galactosamine

Benzyl-N-acetyl-D-galactosaminide (400 mg) was treated with benzaldehyde dimethylacetal to give 450 mg (88%) of 4,6-O-benzylidene derivative. O-Sulphation was achieved with the sulphur trioxide-triethylamine complex in N,N-dimethylformamide yielding 431,3 mg (90%) of 3-O-sulpho-derivative from 400 mg of starting material. The product (350 mg) was catalytically hydrogenated in ethanol-water in presence of palladium hydroxide giving 200 mg (91%) of 3-O-sulpho--N-acetyl-galactosamine. SO calcd. 32%, found 30,2%.

Example 6

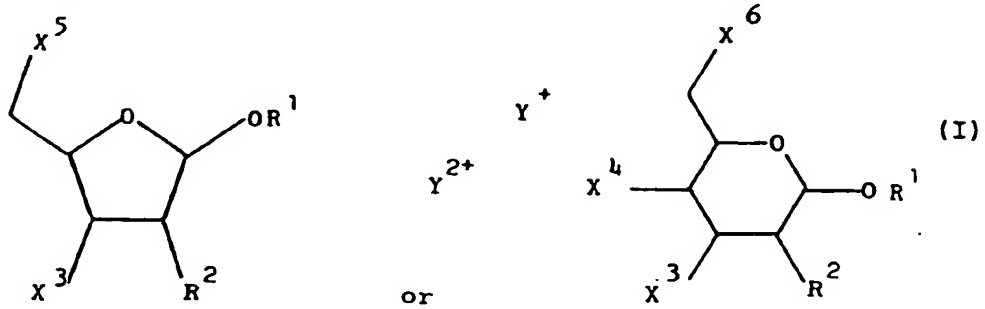
6-O-sulpho-N-acetyl-D-galactosamine

Benzyl-N-acetyl-D-galactosaminide (300 mg) was stirred with trityl chloride (300 mg) and silver nitrate (250 mg) in pyridine (5 ml) at room temperature for 24 hours. The reaction mixture was partitioned between dichloromethane and water and the organic layer was concentrated. Column chromatography of the residue (dichloromethane-methanol (9:1)) gave 6-O-trityl derivative (373mg, 70%).

The product was benzylated as described earlier to obtain 3,4-di-O-benzyl derivative. To remove the 6-O-trityl group this derivative was treated with trifluoro acetic acid (10 ml 1 % in dichloromethane) at room temperature for 20 hours. Column chromatography of the residue on silica gel gave 3,4-di-O-benzyl-6-OH derivative (284mg, 90%). The 3,4-di-O-benzyl-6-OH derivative was O-sulphated as described in example 1 to give 6-O-sulpho-N-acetyl-D-galactosamine (177mg, 92%). SO calc. 32.0% found 30.3%.

Claims

1. A method for preparing mono-, di-, tri-or tetra-sulphate esters of pentoses and hexoses, and salts there of, according to formulas (I)



(I)

where

R^1 is hydrogen, C_1-C_6-alkyl, benzyl, amino acid, nucleotide, or polypeptide;

R^2 is OH, SO_4^- or NHR^3;

R^3 is hydrogen, C_1-C_6-alkyl, acetyl or C_2-C_{24}-acyl, aminoacyl or sulphonyl;

X^3 is OH or SO_4^-;

X^4 is OH or SO_4^-;

X^5 is OH or SO_4^-;

X^6 is OH or SO_4^-;

Y^+, Y^{2+} is H, Na, K, Ca, Zn, Mg, Li, Ba, Mn, Hg, Ag or Au,

characterized in that the sulphation step include treatment with sulphur trioxide-triethylamine complex.

2. A method according to claim 1, **characterized** in that the solvent used in the sulphation step is N,N-dimethylformamide.

3. A method according to claim 1 or 2, **characterized** in that the reaction temperature of the sulphation step is in the range from 20 to 80°C, preferably from 30

to 70°C, most preferably from 40 to 60°C.

4. A method according to any of the claims 1-3, **characterized** in that the method comprises a catalytic hydrogenation step.

5. A method according to claim 4, **characterized** in that the catalyst is palladium hydroxide.

6. A method according to claim 5, **characterized** in that method comprises a protection step, wherein hydroxyl groups of the sugar is protected before the sulphation step.

7. A method according to any of the claims 1-6, **characterized** in that the pentoses and hexoses are selected from the group consisting of ribose, xylose, arabinose, galactose, glucose or mannose.

8. A method according to any of the claims 1-7, **characterized** in that the pentoses and hexoses have an amine-group in position 2.

9. A method according to claim 8, **characterized** in that the prepared hexosamine is a mono-, di-or tri-sulphate substituted N-acetyl-D-galactosamine derivative.

10. A method according to claim 8, **characterized** in that the prepared hexosamine is a mono-, di-or tri-sulphate substituted N-acetyl-D-glucosamine derivative.

11. A method according to claim 8, **characterized** in that the prepared pentosamine is a mono-or di-sulphate substituted N-acetyl-D-ribosamine derivative.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 96/00824

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07H 5/06
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07C, C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, IFIPAT, CA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	Carbohydr. Chem., Volume 7, No 3, 1988, I.G. Leder, "Synthesis of the 3-O, 4-O and 6-O Sulfates of Methyl 2-Amino-2-deoxy-alpha-D-glucopyranoside" page 583 - page 592 --	1-11
X,Y	Carbohydr. Res., Volume 93, 1981, P.J. Archbald et al, "13C-NMR Studies of D-glucose and D-galactose Monosulphates" page 177 - page 190 --	1-11
Y	US 4992533 A (RIKAGAKU KENKYUSHO, MECT CORP.), 12 February 1991 (12.02.91) --	1-11

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 Sept 1996	01 -10- 1996
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Teddy Ercegovic Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 96/00824

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5385891 A (TANABE SEIYAKU CO., LTD), 31 January 1995 (31.01.95) --	1-11
A	US 5116821 A (THE PROCTER & GAMBLE COMPANY), 26 May 1992 (26.05.92) --	1-11

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

05/09/96

International application No.
PCT/SE 96/00824

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A- 4992533	12/02/91	AU-B-	610760	23/05/91
		AU-A-	4231689	05/04/90
		CA-A-	1328450	12/04/94
		EP-A-	0361490	04/04/90
		JP-A-	2096588	09/04/90
US-A- 5385891	31/01/95	CA-A-	2075850	01/03/93
		EP-A-	0531016	10/03/93
		JP-A-	5112601	07/05/93
US-A- 5116821	26/05/92	NONE		

Form PCT/ISA/210 (patent family annex) (July 1992)